

### Listing of Claims:

This listing of claims reflects all claim amendments and replaces all prior versions, and listings, of claims in the application (material to be inserted in amended claims is in underline, and material to be deleted is in ~~strikeout~~).

1.-53. (Cancelled)

54. (Currently amended) A ~~sensing device for sensing an analyte of interest, the device~~ comprising:

a column; and

a first population of beads having a standardized, known surface occupancy immobilized within a first region of the column;

wherein the first populations of beads comprises:

a first type of biomolecule bound to each bead in the first population; and

a first type of fluorescent tag bound to each biomolecule; and

a second population of beads having a standardized, known surface occupancy immobilized within a second region in the column, the second subset of beads comprising:

a second type of biomolecule bound to each bead in the second population; and

a second type of fluorescent tag bound to each biomolecule in the second population;

wherein the first and second regions are distinct from each other; and

wherein the first and second types of biomolecules are not found in the same population of beads.

55. (Previously presented) The device of claim 54 wherein the populations of beads in the column are positioned such that the analyte is initially exposed to only the first population of beads and, as the analyte travels through the column, the analyte is subsequently exposed to only the second population of beads.

56. (Previously presented) The device of claim 53 wherein the first and second populations

of beads are packed into the column such that the second population of beads is layered over the first population of beads.

57. (Previously presented) The device of claim 55 wherein the column includes an obstructive feature configured to separate the first population of beads from the second population of beads.

58. (Previously presented) The device of claim 54 wherein the first and second fluorescent tags initially have an identified pre-complexing fluorescent signature; and, upon binding between the first or second biomolecule and an analyte, the first or second fluorescent tag associated with the analyte-bound biomolecule has a detectable post-complexing fluorescent signature.

59. (Previously presented) The device of claim 58 wherein the pre-complexing fluorescent signature is a first fluorescent emission spectrum and the post-complexing fluorescent signature is second, different, fluorescent emission spectrum.

60. (Previously presented) The device of claim 58 wherein the pre-complexing fluorescent signature is a first lifetime measurement and the post-complexing fluorescent signature is a second lifetime measurement.

61. (Previously presented) The device of claim 54 wherein the first biomolecule comprises the amino acid sequence DYKDDDDK.

62. (Previously presented) The device of claim 54 wherein the first biomolecule is selected from the group consisting of: an antibody, an antigen, a protein, a DNA sequence, an RNA sequence, a peptide and a carbohydrate.

63. (Currently amended) A microfluidic sensing device for sensing an analyte of interest, the device comprising:

a plurality of microfluidic channels wherein at least one microfluidic channel comprises:

a first population of beads having a standardized, known surface occupancy, the first population of beads comprising:

a first type of biomolecule bound to each bead in the first population of beads;

and

a first type of fluorescent tag bound to each biomolecule in the first population of beads;

a second population of beads having a standardized, known surface occupancy, the second population of beads being arranged within the microfluid channel such that an analyte introduced into the microfluidic device is initially exposed to the first population of beads and then subsequently exposed to the second population of beads, the second population of beads comprising:

a second type of biomolecule bound to each bead in the second population of beads; and

a second type of fluorescent tag bound to each biomolecule in the second population of beads; and

wherein the first and second types of biomolecules are not found in the same population of beads.

64. (Previously presented) The microfluidic device of claim 63 comprising at least two channels and wherein the second channel comprises at least one biomolecule that is different from the biomolecules in the first channel.

65. (Previously presented) The microfluidic device of claim 63 wherein the beads are packed into the at least one microfluidic channel such that the first population of beads is layered over the second population of beads.

66. (Previously presented) The microfluidic device of claim 65 wherein the first and second populations of beads are separated by an obstructive feature.

67. (Previously presented) The microfluidic device of claim 63 further comprising a single entry port for analyte delivery.

68. (Previously presented) The microfluidic device of claim 63 further comprising multiple entry ports for analyte delivery, wherein each entry port correlates to a single microfluidic channel.

69. (Withdrawn) A method comprising:  
providing a microcolumn comprising:

an entry port;

a first population of beads immobilized within a first location in the column,

wherein first population of beads includes:

a first biomolecule bound to each bead; and

a first fluorescent tag bound to the biomolecule;

wherein:

the first fluorescent tag initially has an identified pre-complexing fluorescent signature; and

upon binding between the first biomolecule and an analyte, the first fluorescent tag has a detectable post-complexing fluorescent signature;

a second population of beads immobilized within a second, distinct, location in the column, wherein second population of beads includes:

a second biomolecule bound to each bead; and

a second fluorescent tag bound to the biomolecule;

wherein:

the second fluorescent tag initially has an identified pre-complexing fluorescent signature; and

upon binding between the second biomolecule and an analyte, the second fluorescent tag has a detectable post-complexing fluorescent signature;

delivering an analyte to the microcolumn via the entry port; and

detecting the difference between the post-complexing fluorescent signature of the first or second fluorescent tag and the pre-complexing fluorescent signature of the first or second fluorescent tag.

70. (Withdrawn) The method of claim 69 wherein the analyte can be unbound from the first and second biomolecules such that the column can be reused.

71. (Withdrawn) The method of claim 70 wherein the first and second biomolecules are selected such that they will only bind analyte in the presence of a first substance, the method further comprising;

introducing analyte to the column in the presence of a first substance;

detecting the difference between the post-complexing fluorescent signature of the first or second fluorescent tag and the pre-complexing fluorescent signature of the first or second fluorescent tag.

72. (Withdrawn) The method of claim 71 further comprising depleting the system of the first substance such that the analyte is released by the first and second molecules and can be flushed from the system.

73. (Withdrawn) The method of claim 69 wherein the first biomolecule and first fluorescent tag are selected to perform a first assay on the analyte and the second biomolecule and second fluorescent tag are selected to perform a second assay on the analyte.

74. (New) The device of claim 54 wherein each bead contains on the order of 10 million binding sites.

75. (New) The device of claim 54 wherein each bead contains greater than 2 million receptors.

76. (New) The microfluidic device of claim 63 wherein each bead contains on the order of 10 million binding sites.

77. (New) The microfluidic device of claim 63 wherein each bead contains greater than 2 million receptors.